



# SEQUENCE LISTING

<110> Genencor International, Inc.  
Poulose, Ayrookaran J.  
Estell, David A.  
Kellis, Jr., James  
Bott, Richard R.

<120> Multiply-Substituted Protease Variants

<130> GC716-2-PCT

<140> PCT/US03/01448

<141> 2003-01-16

<150> US 60/350,222

<151> 2002-01-16

<160> 10

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 1494

<212> DNA

<213> Bacillus amyloliquefaciens

<400> 1

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gcacgatgag	cgccgctaag	aagaaagatg	tcattttctga	aaaaggcggg	aaagtgcata	300
agcaattcaa	atatgtagac	gcagcttcag	ctacattaaa	cgaaaaagct	gtaaaaaat	360
tgaaaaaaga	cccagcgcgc	gcttacggtg	aagaagatca	cgtagcacat	gcgtacgcgc	420
agtccgtgcc	ttacggcgta	tcacaaaatta	aagcccctgc	tctgcaactct	caaggctaca	480
ctggatcaaa	tgtaaagta	gcggttatcg	acagcgggat	cgattcttct	catcctgatt	540
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gccaatacag	ctggatcatt	aacggaatcg	agtgggcgat	cgcaacaat	atggacgtta	780
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caagcacagt	gggctaccct	ggtaaatacc	cttctgtcat	tgcagtaggc	gctgttgaca	960
gcagcaacca	aagagcatct	ttctcaagcg	taggacctga	gcttgatgtc	atggcacctg	1020
gcgtatctat	ccaaagcacg	cttcctggaa	acaaatacgg	ggcgtaacaac	ggtacgtcaa	1080
tggcatctcc	gcacgttgcc	ggagcggctg	ctttgattct	ttctaagcac	ccgaactgga	1140
caaacactca	agtccgcagc	agtttagaaa	acaccactac	aaaacttggt	gattctttct	1200
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aacggccaag	tcctgaaaac	tctcaatcgc	cgcttcccgg	tttccgggtc	gctcaatgcc	1440
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<210> 2

<211> 382  
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 <213> Bacillus amyloliquefaciens

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 <223> Xaa = Pro or Asn

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 <223> Xaa = Glu or Gln

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 Ile Phe Thr Met Ala Phe Gly Ser Thr Ser Ser Ala Gly Ala Ala Gly  
 20 25 30  
 Lys Ser Asn Gly Glu Lys Lys Tyr Ile Val Gly Phe Lys Gln Thr Met  
 35 40 45  
 Ser Thr Met Ser Ala Ala Lys Lys Lys Asp Val Ile Ser Glu Lys Gly  
 50 55 60  
 Gly Lys Val Gln Lys Gln Phe Lys Tyr Val Asp Ala Ala Ser Ala Thr  
 65 70 75 80  
 Leu Asn Glu Lys Ala Val Lys Glu Leu Lys Lys Asp Pro Ser Val Ala  
 85 90 95  
 Tyr Val Glu Glu Asp His Val Ala His Ala Tyr Ala Gln Ser Val Pro  
 100 105 110  
 Tyr Gly Val Ser Gln Ile Lys Ala Pro Ala Leu His Ser Gln Gly Tyr  
 115 120 125  
 Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp Ser Gly Ile Asp Ser  
 130 135 140  
 Ser His Pro Asp Leu Lys Val Ala Gly Gly Ala Ser Met Val Pro Ser  
 145 150 155 160  
 Glu Thr Xaa Xaa Phe Gln Asp Xaa Asn Ser His Gly Thr His Val Ala  
 165 170 175  
 Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly Val Leu Gly Val Ala  
 180 185 190  
 Pro Ser Xaa Xaa Leu Tyr Ala Val Lys Val Leu Gly Xaa Xaa Gly Ser  
 195 200 205  
 Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu Trp Ala Ile Ala Asn

210	215	220
Asn Met Asp Val Ile	Asn Met Ser Leu Gly Gly	Pro Ser Gly Ser Ala
225	230	235
Ala Leu Lys Ala Ala	Val Asp Lys Ala Val Ala	Ser Gly Val Val Val
245	250	255
Val Ala Ala Ala Gly	Asn Glu Gly Xaa Xaa Gly	Ser Ser Ser Thr Val
260	265	270
Gly Tyr Pro Gly Lys Tyr	Pro Ser Val Ile Ala Val	Gly Ala Val Asp
275	280	285
Ser Ser Asn Gln Arg Ala	Ser Phe Ser Ser Val	Gly Pro Glu Leu Asp
290	295	300
Val Met Ala Pro Gly Val	Ser Ile Gln Ser Thr Leu	Pro Gly Asn Lys
305	310	315
Tyr Gly Ala Tyr Asn Gly	Thr Ser Met Ala Ser Pro	His Val Ala Gly
325	330	335
Ala Ala Ala Leu Ile Leu	Ser Lys His Pro Asn Trp	Thr Asn Thr Gln
340	345	350
Val Arg Ser Ser Leu Xaa	Asn Thr Thr Thr Lys Leu	Gly Asp Ser Phe
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Tyr Tyr Gly Lys Gly Leu	Ile Asn Val Gln Ala Ala	Ala Gln
370	375	380

<210> 3  
 <211> 275  
 <212> PRT  
 <213> Bacillus amyloliquefaciens

<400> 3

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His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp	20	25	30	
Ser Gly Ile Asp Ser Ser His Pro Asp Leu Lys Val Ala Gly Gly Ala	35	40	45	
Ser Met Val Pro Ser Glu Thr Asn Pro Phe Gln Asp Asn Asn Ser His	50	55	60	
Gly Thr His Val Ala Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly	65	70	75	80
Val Leu Gly Val Ala Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu	85	90	95	
Gly Ala Asp Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu	100	105	110	
Trp Ala Ile Ala Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly	115	120	125	
Pro Ser Gly Ser Ala Ala Leu Lys Ala Ala Val Asp Lys Ala Val Ala	130	135	140	
Ser Gly Val Val Val Val Ala Ala Ala Gly Asn Glu Gly Thr Ser Gly	145	150	155	160
Ser Ser Ser Thr Val Gly Tyr Pro Gly Lys Tyr Pro Ser Val Ile Ala	165	170	175	
Val Gly Ala Val Asp Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Val	180	185	190	
Gly Pro Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr	195	200	205	
Leu Pro Gly Asn Lys Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Ser	210	215	220	
Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn				

225                      230                      235                      240  
 Trp Thr Asn Thr Gln Val Arg Ser Ser Leu Glu Asn Thr Thr Thr Lys  
                                  245                      250                      255  
 Leu Gly Asp Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala  
                                  260                      265                      270  
 Ala Ala Gln  
                                  275

<210> 4  
 <211> 275  
 <212> PRT  
 <213> *Bacillus subtilis*

<400> 4  
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 1                      5                      10                      15  
 His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp  
                                  20                      25                      30  
 Ser Gly Ile Asp Ser Ser His Pro Asp Leu Asn Val Arg Gly Gly Ala  
                                  35                      40                      45  
 Ser Phe Val Pro Ser Glu Thr Asn Pro Tyr Gln Asp Gly Ser Ser His  
                                  50                      55                      60  
 Gly Thr His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly  
 65                      70                      75                      80  
 Val Leu Gly Val Ser Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu  
                                  85                      90                      95  
 Asp Ser Thr Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu  
                                  100                      105                      110  
 Trp Ala Ile Ser Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly  
                                  115                      120                      125  
 Pro Thr Gly Ser Thr Ala Leu Lys Thr Val Val Asp Lys Ala Val Ser  
                                  130                      135                      140  
 Ser Gly Ile Val Val Ala Ala Ala Ala Gly Asn Glu Gly Ser Ser Gly  
 145                      150                      155                      160  
 Ser Thr Ser Thr Val Gly Tyr Pro Ala Lys Tyr Pro Ser Thr Ile Ala  
                                  165                      170                      175  
 Val Gly Ala Val Asn Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Ala  
                                  180                      185                      190  
 Gly Ser Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr  
                                  195                      200                      205  
 Leu Pro Gly Gly Thr Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Thr  
                                  210                      215                      220  
 Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Thr  
 225                      230                      235                      240  
 Trp Thr Asn Ala Gln Val Arg Asp Arg Leu Glu Ser Thr Ala Thr Tyr  
                                  245                      250                      255  
 Leu Gly Asn Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala  
                                  260                      265                      270  
 Ala Ala Gln  
                                  275

<210> 5  
 <211> 274  
 <212> PRT  
 <213> *Bacillus licheniformis*

<400> 5

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Gln	Ala	Gln	Gly	Phe	Lys	Gly	Ala	Asn	Val	Lys	Val	Ala	Val	Leu	Asp
			20					25					30		
Thr	Gly	Ile	Gln	Ala	Ser	His	Pro	Asp	Leu	Asn	Val	Val	Gly	Gly	Ala
		35					40					45			
Ser	Phe	Val	Ala	Gly	Glu	Ala	Tyr	Asn	Thr	Asp	Gly	Asn	Gly	His	Gly
	50				55					60					
Thr	His	Val	Ala	Gly	Thr	Val	Ala	Ala	Leu	Asp	Asn	Thr	Thr	Gly	Val
65					70					75					80
Leu	Gly	Val	Ala	Pro	Ser	Val	Ser	Leu	Tyr	Ala	Val	Lys	Val	Leu	Asn
				85					90					95	
Ser	Ser	Gly	Ser	Gly	Ser	Tyr	Ser	Gly	Ile	Val	Ser	Gly	Ile	Glu	Trp
			100					105					110		
Ala	Thr	Thr	Asn	Gly	Met	Asp	Val	Ile	Asn	Met	Ser	Leu	Gly	Gly	Ala
		115					120					125			
Ser	Gly	Ser	Thr	Ala	Met	Lys	Gln	Ala	Val	Asp	Asn	Ala	Tyr	Ala	Arg
	130					135					140				
Gly	Val	Val	Val	Val	Ala	Ala	Ala	Gly	Asn	Ser	Gly	Asn	Ser	Gly	Ser
145					150					155					160
Thr	Asn	Thr	Ile	Gly	Tyr	Pro	Ala	Lys	Tyr	Asp	Ser	Val	Ile	Ala	Val
			165						170					175	
Gly	Ala	Val	Asp	Ser	Asn	Ser	Asn	Arg	Ala	Ser	Phe	Ser	Ser	Val	Gly
		180						185					190		
Ala	Glu	Leu	Glu	Val	Met	Ala	Pro	Gly	Ala	Gly	Val	Tyr	Ser	Thr	Tyr
	195						200					205			
Pro	Thr	Asn	Thr	Tyr	Ala	Thr	Leu	Asn	Gly	Thr	Ser	Met	Ala	Ser	Pro
	210					215					220				
His	Val	Ala	Gly	Ala	Ala	Ala	Leu	Ile	Leu	Ser	Lys	His	Pro	Asn	Leu
225					230					235					240
Ser	Ala	Ser	Gln	Val	Arg	Asn	Arg	Leu	Ser	Ser	Thr	Ala	Thr	Tyr	Leu
			245					250						255	
Gly	Ser	Ser	Phe	Tyr	Tyr	Gly	Lys	Gly	Leu	Ile	Asn	Val	Glu	Ala	Ala
			260					265					270		

Ala Gln

<210> 6

<211> 269

<212> PRT

<213> Bacillus lentus

<400> 6

Ala	Gln	Ser	Val	Pro	Trp	Gly	Ile	Ser	Arg	Val	Gln	Ala	Pro	Ala	Ala
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His	Asn	Arg	Gly	Leu	Thr	Gly	Ser	Gly	Val	Lys	Val	Ala	Val	Leu	Asp
			20					25					30		
Thr	Gly	Ile	Ser	Thr	His	Pro	Asp	Leu	Asn	Ile	Arg	Gly	Gly	Ala	Ser
		35				40					45				
Phe	Val	Pro	Gly	Glu	Pro	Ser	Thr	Gln	Asp	Gly	Asn	Gly	His	Gly	Thr
	50				55					60					
His	Val	Ala	Gly	Thr	Ile	Ala	Ala	Leu	Asn	Asn	Ser	Ile	Gly	Val	Leu
65					70					75					80
Gly	Val	Ala	Pro	Ser	Ala	Glu	Leu	Tyr	Ala	Val	Lys	Val	Leu	Gly	Ala
			85					90					95		
Ser	Gly	Ser	Gly	Ser	Val	Ser	Ser	Ile	Ala	Gln	Gly	Leu	Glu	Trp	Ala
			100					105					110		

Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser  
           115                          120                          125  
 Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly  
           130                          135                          140  
 Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser  
 145                          150                          155                          160  
 Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln  
                           165                          170                          175  
 Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile  
                           180                          185                          190  
 Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr  
                           195                          200                          205  
 Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala  
           210                          215                          220  
 Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile  
 225                          230                          235                          240  
 Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu  
                           245                          250                          255  
 Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg  
                           260                          265

<210> 7  
 <211> 27  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> primer

<400> 7  
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27

<210> 8  
 <211> 27  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> primer

<400> 8  
 gtgtgtgggc cctattcgga tattgag

27

<210> 9  
 <211> 275  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> consensus sequence

<221> VARIANT  
 <222> (1)...(275)  
 <223> Xaa = Any Amino Acid

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